



Nyctomyia biunguiculata, a new cavernicolous species of tribe Aedini (Diptera: Culicidae) from southern Thailand

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Abstract

Nyctomyia biunguiculata, **sp. n.**, is described from a unique male reared from a pupa (exuviae partially decomposed and over cleared) collected from a rimstone pool deep inside Wat Sumano cave located in Phatthalung Province of southern Thailand. The genitalia of the holotype are illustrated, and the species is compared with *Nc. pholeocola*, the type species of the genus described from a cave in another province of southern Thailand. The species has not been found outside the cave and appears to be a true troglobite.

Key words: *Nyctomyia biunguiculata* **sp. n.**, *Nc. pholeocola*, mosquito, taxonomy, troglobite

Introduction

Caves harbour a number of distinct and interesting mosquito species. These are true cavernicolous species that are restricted to specific caves and have no apparent close relatives in the outside environment. They seemingly evolved from ancestors which had a propensity for entering caves in search of food sources, used cavernicolous water bodies for oviposition and gave rise to isolated cave-dwelling populations that evolved independently from the epigeal parental stocks. These species became troglobites, i.e. they only live in caves and do not leave them (Chapman, 1982). Unlike other troglofauna, cavernicolous mosquitoes do not seem to have evolved any obvious physical adaptations to life in the subterranean environment, such as loss of eyes or pigmentation. However, it is likely that they have evolved physiological adaptations and characteristics for continued survival underground.

Seven apparently true troglobite mosquito species are known, including *Anopheles* (*Cellia*) *hamoni* Adam (DR Congo), *An. (Cel.) vanhoofti* Wanson & Lebiec (DR Congo), *Borichinda cavernicola* Rattananarithikul & Harbach (Thailand), *Culex* (*Culiciomyia*) *harrisoni* Sirivanakarn (Thailand), *Isoaedes cavaticus* (Reinert) (Thailand), *Nyctomyia pholeocola* (Linton & Harbach) (Thailand) and *Uranotaenia* (*Pseudoficalbia*) *cavernicola* Mattingly (Belgian Congo). Two species, *Anopheles* (*Cellia*) *smithii* Theobald (northwestern Africa) and *Ur. (Pfc.) sumethi* Peyton & Rattananarithikul (Thailand), are known to enter caves, and two others, *An. (Cel.) caroni* Adam (DR Congo) and *Culex* (*Lophoceraomyia*) *kusaiensis* Bohart (Caroline Islands), have been found at the entrances (mouths) of caves. The new species described here appears to be another troglobite.

Material and methods

This study is based on a male mosquito reared from a pupa collected from a rimstone pool in Wat Sumano cave located in Phatthalung Province of southern Thailand (see **Holotype**). The specimen was point-mounted on an insect pin and its genitalia were dissected, cleared in 5% NaOH for 2 h at 50°C and mounted in Euparal on a microscope slide. The pinned specimen was examined under simulated natural light with an Olympus SZ6045

stereomicroscope and its genitalia were studied and illustrated with an Olympus BX50 compound microscope fitted with differential interference contrast optics and a camera lucida. The associated pupal exuviae was mounted in Euparal on a microscope slide but it is in very poor condition (partially decomposed and over cleared) and could not be studied. The morphological terminology used herein is listed and defined in the Anatomical Glossary of the Mosquito Taxonomic Inventory (<http://mosquito-taxonomic-inventory.info/>).

Taxonomy

Nyctomyia biunguiculata Harbach, sp. n.

Male. Medium-sized mosquito. Dark scaling light to dark brown, pale scaling white to golden. *Head:* Vertex with large falcate scales and few broad scales laterally, ocular and interocular areas with smaller falcate scales, erect scales slender, golden, borne on occiput and posterior area of vertex; eyes narrowly separated above antennae, 2 prominent interocular setae present; antenna shorter than proboscis, length about 1.75 mm, pedicel and flagellomere 1 with few small spatulate scales on mesal surface, flagellar whorls comprised of numerous long strongly developed setae projecting mainly dorsally and ventrally, flagellomeres 12 and 13 very long, combined length about 0.4 length of flagellum; maxillary palpus slender, about 0.3 length of proboscis, comprised of 4 palpomeres, fourth vestigial, dorsal and lateral surfaces dark-scaled, ventral surface without scales, apex of palpomere 3 with few short setae; proboscis long and slender, length (including labella) about 2.3 mm, distinctly longer than forefemur, about 1.25 times as long. *Thorax:* Integument light brown, darker dorsally. Scutum with pattern of coarse pale scales on background of fine dark scales, pale scaling as follows: acrostichal line bifurcating posteriorly into lateral prescutellar patches, anterior patch on scutal fossa contiguous with anterior dorsocentral line, posterior scutal fossal line joined to posterior dorsocentral line, and narrow lateral patch extending from prescutal suture onto margin of supraalar area; acrostichal and dorsocentral setae weakly developed, inconspicuous; scutellum with pale falcate scales on mid and lateral lobes; paratergite narrow, without scales and setae; antepronotum with large pale falcate scales, postpronotum with few pale falcate scales on upper anterior corner; brown setae on antepronotum and upper posterior margin of postpronotum; postspiracular setae present; pleura with golden to golden-brown setae on upper proepisternum (8), prealar area (7), upper (7) and lower (7) mesokatepisternum (in more or less continuous row), and upper (9) and lower (3) mesepimeron; pale spatulate scales on upper proepisternum, postspiracular area, upper and lower mesokatepisternum, and upper anterior mesepimeron between upper and lower mesepimeral setae. *Wing:* Length about 2.8 mm, width about 0.5 mm; entirely dark-scaled, veins with few scales, tertiary fringe scales absent; remigium without setae; alula with narrow scales on margin. *Halter:* Integument pale, capitellum darker dorsally with faint pale scales. *Legs:* Anterolateral surface of forecoxa with pale spatulate scales and prominent brown setae, midcoxa with pale spatulate scales on anterior side of mid-lateral row of prominent brown setae, hindcoxa without scales, with posterolateral row of prominent brown setae; femora with narrow indistinct apical pale knee spots, forefemur length about 1.8 mm; tibiae and tarsi dark-scaled; tarsomere 5 of both forelegs missing but foreungues probably like midungues (*vide* Harbach *et al.*, 2007), midungues enlarged, anterior and posterior unguis of each with a tooth, hindungues small, equal, simple. *Abdomen:* Tergum I with median dorsal patch of pale and dark scales, laterotergite with patch of white spatulate scales; terga II–VII with relatively narrow straight basal pale bands, tergum VIII (post-rotation ventral position) mainly dark-scaled, posterior margin straight; sterna II–VII with basal pale bands, bands nearly 0.5 length of respective sternum, sternum VIII (post-rotation dorsal position) mainly pale-scaled, posterior margin straight. *Genitalia* (Fig. 1): Tergum IX lobes poorly developed, separated, each with row of small setae along posterior margin; sternum IX (not illustrated) about as long as broad, with median pair of setae about 0.75 from anterior margin; gonocoxite elongate, mesal membrane developed to apex, basal dorsomesal lobe slightly produced with dense cluster of relatively long setae, dorsal surface with sparse short setae, lateral and ventral surfaces with long setae and spatulate scales, apicodorsal lobe not developed; gonostylus long, about 0.70 length of gonocoxite, slender at base, expanded laterally in distal 0.45, proximal portion with few widely spaced ventromesal setae, expanded portion with uneven lateral line of close-set simple setae, some longer ventromesal setae and a slightly subapical pair of stout gonostylar claws, claws about 0.1 length of gonostylus; claspette slender, apex with 2 stout relatively short setae and 1 or 2 slender setae; proctiger well developed, about 0.3 length of gonocoxite, paraproct

tapered and bent tergal, without apical teeth; cercal sclerite faintly developed (not illustrated), more or less membranous, fused with paraproct, cercal setae absent; tergum X distinct; aedeagus relatively large, located mostly anterior to proctiger in resting position, lateral plates bent tergal, joined distally, apical tergo-lateral margin of each with line of short ridge-like teeth.

Female, egg, larva and pupa. Unknown.

Etymology. Latin, *bi-* (two or double) and *unguiculata* (with a small claw), literally meaning “double little claw”, in reference to the pair of gonostylar claws of the male genitalia.

Bionomics. The holotype male of *Nc. biunguiculata* was reared from a pupa collected from a rimstone pool (0.5 x 0.5 m, depth 35 cm) located 50 m inside Wat Sumano cave, Srinagarindra District, in Phatthalung Province of southern Thailand. The water in the pool was clear, cold and devoid of vegetation and plant matter. The cave harbours numerous bats that are likely to be the primary source of food for adult females. The species has not been found outside the cave.

Distribution. *Nyctomyia biunguiculata* is only known from the Wat Sumano cave in Phatthalung Province of southern Thailand (Fig. 2).

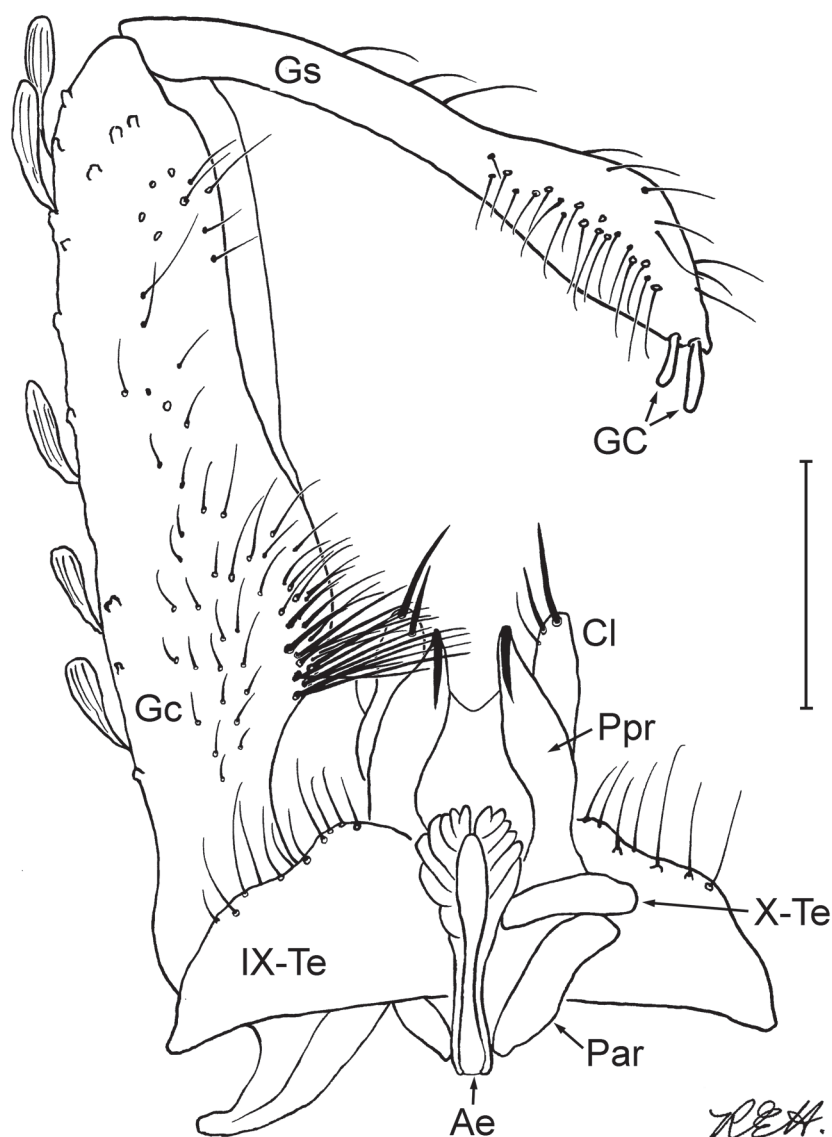


FIGURE 1. Male genitalia of *Nyctomyia biunguiculata* (tergal view), right gonocoxopodite not drawn and mid-section of tergum IX omitted to show internal structures. Ae = aedeagus; Cl = claspette; Gc = gonocoxite; GC = gonostylar claws; Gs = gonostylus; Par = paramere; Ppr = paraproct. Scale bar = 0.1 mm.



FIGURE 2. Outline map of Thailand showing the locations (black dots) of Wat Tham Panthurat, type locality of *Nc. pholeocola*, in Surat Thani Province (1), and Wat Sumano cave, type locality of *Nc. biunguiculata*, in Phatthalung Province (2). The type localities of the other Thai troglobite species are indicated as follows: ▲ = *Culex harrisoni* (Chiang Dao cave, Chiang Dao District, Chiangmai Province); ♦ = *Borichinda cavernicola* (Borichinda cave, Chomthong District, Chiangmai Province); ♥ = *Isoaedes cavaticus* (Gang Lawa cave, Sai Yok District, Kanchanaburi Province).

Holotype. Male, with pupal exuviae (partially decomposed and over cleared) and dissected genitalia on separate microscope slides, THAILAND: *Phatthalung Province*, Srinagarindra District, Wat Sumano cave (07° 35' 10.4" N, 99° 52' 6.5" E), rimstone pool (0.5 x 0.5 m), 15.iv.2011 (M. Isenstadt). Deposited in the Natural History Museum, London.

Discussion

Three mosquito species, each the type species of a monobasic genus of tribe Aedini, were previously discovered in caves in Thailand, including *Borichinda cavernicola* (type locality: Borichinda cave, Chiangmai Province, northern Thailand), *Isoaedes cavaticus* (type locality: Gang Lawa cave, Kanchanaburi Province, west-central Thailand) and *Nyctomyia phleocola* (type locality: Wat Tham Phanturat cave, Surat Thani Province, southern Thailand) (Harbach *et al.*, 2007; Reinert, 1979; Harbach *et al.*, 2013, respectively). *Isoaedes cavaticus* has also been found in two caves located about 120 km northwest of the type locality (Harrison *et al.*, 1991). A fourth species, *Culex harrisoni* of subgenus *Culiciomyia*, is only known from Chiang Dao cave in the mountainous area of Chiang Dao District, Chiangmai Province, northern Thailand (Sirivanakarn, 1977). The new species described here is the first newly discovered troglobite species to be placed in an existing generic-level taxon. Despite being found in a cave located about 450 km southeast of the cave that harbours *Nc. phleocola* (Fig. 2, see also for the type localities of the other Thai cave species), there is no doubt that these species are closely related members of the same genus. The male of *Nc. biunguiculata* exhibits the generic characteristics of *Nyctomyia* (see Harbach *et al.*, 2013, as *Nyx*, preoccupied) and differs from the male of *Nc. phleocola* by being slightly larger and in having narrow scales on the alula of the wing (scales broad in *Nc. phleocola*), more numerous setae on the basal dorsomesal lobe of the gonocoxite (not apparent in Fig. 1), expanded distal portion of the gonostylus with an uneven row of relatively short, straight setae (straight row of longer setae with hooked apices in *Nc. phleocola*); claspette with two shorter, stouter setae (two longer, slender setae in *Nc. phleocola*), several setae on the proximal part of the gonostylus (absent in *Nc. phleocola*) and two slightly subapical gonostylar claws (single apical claw in *Nc. phleocola*). Additionally, there is an indication that the aedeagus is slightly larger and borne in a more cephalad position relative to the proctiger in *Nc. unguiculata*.

Prior to the discovery of *Nc. biunguiculata*, the mosquito fauna of Thailand included 460 species classified in 54 genera and 40 subgenera (Rattarithikul *et al.*, 2010; Harbach *et al.*, 2013) [the preoccupied generic name proposed by the latter authors was replaced by Harbach, 2013]. With the addition of *Nc. biunguiculata*, the Thai mosquito fauna now includes 461 species. The number of species in Thailand is certainly greater than this as several undescribed species are currently known and more are likely to be discovered, especially in the country's numerous caves.

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References

- Chapman (1982) The origins of troglobites. *Proceedings of the University of Bristol Spelaeological Society*, 16, 133–141.
- Harbach, R.E. (2013) *Nyctomyia*, a replacement name for the preoccupied genus *Nyx* Harbach & Linton, 2013 (Diptera: Culicidae). *Zootaxa*, 3691 (1), 1–199.
<http://dx.doi.org/10.11646/zootaxa.3691.1.9>
- Harbach, R.E., Kitching, I.J., Culverwell, C.L., Howard, R.M. & Linton, Y.-M. (2013) *Nyx phleocola*, a new genus and cavernicolous species of tribe Aedini (Diptera: Culicidae) from southern Thailand based on morphological and molecular data. *Zootaxa*, 3683, 159–177.
<http://dx.doi.org/10.11646/zootaxa.3683.2.5>
- Harbach, R.E., Rattarithikul, R., Howard, T.M., Linton, Y.-M. & Kitching, I.J. (2007) Systematics of a new genus and

- cavernicolous species of the mosquito tribe Aedini (Diptera: Culicidae) from Thailand. *Proceedings of the Entomological Society of Washington*, 109, 469–488.
- Harrison, B.A., Rattanarithikul, R., Peyton, E.L. & Mongkolpanya, K. (1991) Taxonomic changes, revised occurrence records and notes on the Culicidae of Thailand and neighboring countries. *Mosquito Systematics* (for 1990), 22, 196–227.
- Reinert, J.F. (1979) A description of *Isoaedes*, a new subgenus of *Aedes* Meigen, and its type-species, *Ae. (Isa.) cavaticus* new species (Diptera: Culicidae). *Mosquito Systematics*, 11, 144–162.
- Rattanarithikul, R., Harbach, R.E., Harrison, B.A., Panthusiri, P., Coleman, R.E. & Richardson, J.H. (2010) Illustrated keys to the mosquitoes of Thailand VI. Tribe Aedini. *Southeast Asian Journal of Tropical Medicine and Public Health*, 41 (Supplement 1), 1–225.
- Sirivanakarn, S. (1977) Redescription of four Oriental species of *Culex* (*Culiciomyia*) and the description of a new species from Thailand (Diptera: Culicidae). *Mosquito Systematics*, 9, 93–111.